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THE CULTIVATION OF TUBERCLE BACILLI*

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It is now well over fifty years since the tubercle bacillus was first grown artificially, and it is surprising that the cultivation of tubercle bacilli is still poorly outlined in textbooks so that the initiate in this field frequently has to proceed through the laborious experiences so well illustrated by the history of this subject. Koch originally grew the tubercle bacillus on an inspissated serum medium which today would be considered a poor nutrient for diagnostic purposes, as will be illustrated later. As you well know, Koch succeeded not only in demonstrating the bacilli under the microscope, but he grew them after being able to transmit the disease to animals; and through the animal, eliminated the contaminating and confusing microorganisms which might interfere with successful culture. He was aware of the fact that the animal and human organism ably eliminated contaminations, so that after the development of tuberculous pus or caseous material, and particularly when the bacilli could be seen under the microscope, he was successful in obtaining pure cultures from such material. His patience was well demonstrated when, after the initial attempt with no growth within the first week, he returned the culture tubes to the incubator and finally noted, after several weeks, the appearance of small colonies of dis-

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tinctive bacillary masses. I doubt, however, whether he had a true quantitative conception of the situation at hand, and this is extremely important to an understanding of our present day accomplishments.[1] Before the advent of the twentieth century, we were well aware that the tubercle bacillus was not the only acid-fast microorganism in nature,[2] but that there were numerous acid-fast saprophytic bacilli to be found, and that these could be confusing in the diagnosis of human and bovine tuberculosis. As an illustration, we have named one of these acid-fast saprophytes the "smegma bacillus," and in genito-urinary work, the possible confusion in diagnosis has long been recognized. Of the pathogens, we, of course, now recognize three main varieties of tubercle bacilli, exclusive of the leprosy bacillus, which is also acid-fast; the avian, the bovine, and the human tubercle bacillus. Biologically, the mazes of pathogenicity of these three microorganisms are still unsolved; but practically, we might say that the human and bovine bacilli concern us mainly in human disease, while the avian bacillus is extremely rare as a disease producer in the human body. In my early experiences, I was greatly confused by the lack of quantitative interpretation and was seeking to find that Utopian material which when added to the ordinary nutrients could so enhance these mediums that tubercle bacilli would readily grow upon them.[3] In spite of elaborate and numerous tests with a great many different strains of bacilli, these investigations proved unproductive. The reason for this was probably caused by the fact that we were a little confused as to what we were trying to achieve. However, of this we were certain: that the guinea pig was highly susceptible to both human and bovine tubercle bacilli, and that guinea pigs could be used for determining the presence of the tubercle bacilli in material which would repeatedly reveal none under the microscope. If we could only imitate the guinea pig in delicacy by the use of the culture tube, we should have a valuable and simple diagnostic aid in tuberculosis. We were also aware of the fact that tubercle bacilli could be grown profusely on the simplest of nutrient mediums, such as ordinary laboratory broth or plain glycerol agar. Among the attempts to destroy the contaminants in human pathological material to thus imitate the guinea pig, may be cited the use of materials ranging from formalin through chlorine containing mixtures such as antiformin and Labarraque's solution. All such attempts were reported with variably favorable results, but upon further repetition they did not fulfill the requirement in efficiency established by the use of the guinea pig in diagnosis. In the interim, a good nutrient medium appeared in 1902 in the form of the inspissated whole egg medium of Dorset,[4] developed in the Department of Animal Industry, but its true efficiency was not quantitatively determined, although egg mediums as a whole

were recognized by many students to be highly serviceable for growing tubercle bacilli, and were used in various mixtures. In 1915, Petroff[5] of Saranac Lake devised a method which proved to be the most acceptable recommended up to that time for the isolation of tubercle bacilli from contaminated tuberculous material, embodying the destruction of contaminants by means of sodium hydroxide and an egg nutrient containing a static dye, gentian violet, to prevent the growth of contaminants escaping the action of the sodium hydroxide, and suitable for growing tubercle bacilli. On the basis of subsequent work,[6] we can now analyze this method more carefully. The sodium hydroxide used to destroy the contaminating microorganisms, and satisfactorily so by using an equal volume of a 3% concentration, however, destroyed some of the tubercle bacilli in the specimen. The gentian violet added to the egg medium proved to be a static agent in the concentration of 1:10,000 used and, therefore, retarded the growth of some of the tubercle bacilli. However, this method was a decided advance and suggested possibilities of elucidating this problem. It also attracted attention to the fact that there are actually two practical purposes for which tubercle bacilli are grown, and these might be different and divergent; one, for the purpose of obtaining massive amounts of colonies of the bacilli. This phase is well illustrated by the studies by Long and Seibert[7] from which they developed a synthetic non-protein nutrient medium for the study of tuberculin and coincidently the metabolism of the tubercle bacillus. Since then, it has also lead to an elaborate study of the chemistry of the tubercle bacillus by Johnson and Anderson[8]. It was soon realized, however, that these nutrient mediums, although valuable for obtaining large amounts of bacillary growth and their products of metabolism and disintegration, were of absolutely no value for the purpose of growing small numbers of tubercle bacilli for diagnostic purposes—the second objective in cultivating tubercle bacilli. Since the second phase of this subject is the one that interests us primarily from a diagnostic medical standpoint, I will limit the rest of my discourse to this subject. I regret that time and space will not permit a detailed discussion of this extensive subject here but will limit my presentation only to the important phases of cultivating tubercle bacilli for diagnostic purposes, which is primarily concerned with the successful cultivation from small numbers of bacilli in a pathological specimen such as sputum, urine, or pus. To comprehend the development and significance of this subject, an exact knowledge of the number of tubercle bacilli being dealt with is pertinent; and this can best be understood by knowing the approximate number of bacilli in one milligram of bacillary culture mass.[9] From this as a starting point, one can readily approach the conditions of practice. With this approach, we soon learned that

the microscopic examination of the stained smear had its limitations.[10] Pointedly, the microscopic smear examination requires the presence of more than 10,000 bacilli per cubic centimeter to be discernable at all, while the guinea pig can still be infected with as few as ten bacilli of a virulent strain. This information proved valuable since it indicated that the problem of nutrients had to be carefully studied for tubercle bacilli. In the course of these studies, we disclosed that we could not enhance the growth of small numbers of tubercle bacilli to an appreciable extent by the addition of various and sundry vitamins and split products added to agar or broth agar,[11] and that the tubercle bacillus was as preferential about its nutrient requirements when the bacilli were few in number in a planted specimen as some of the more complex living organisms. This can be seen graphically when we chart the results of planting graded amounts of fine suspensions of tubercle bacilli, starting with one milligram and diluting to the point of extinction of about one-billionth milligram. While 0.1 mg. plantings will grow well only on Long's non-protein nutrient agar and on glycerol agar, 0.000,1 mg. plantings in fine suspension will still grow on the gentian violet egg medium of Petroff. However, the inspissated egg medium of Dorset will readily give positive growth in 0.000,000,1 mg., and on the neutralized autoclaved potato cylinder medium, growth can be obtained to a dilution of one-hundred millionth to one-billionth milligrams. With the potato, we were thus able to obtain positive plants practically to the point of extinction dilution of the bacilli.[12] Later, we found other nutrient mediums such as an inspissated egg yolk to support the growth of plantings of few bacilli.[13]

During the course of our investigations on nutrients, we endeavored to simplify the mediums as far as possible by eliminating unnecessary extraneous materials. Thus, it was found that a good nutrient medium for the tubercle bacillus, such as egg yolk, potato or animal tissues, did not require the addition of the various materials such as beef extract, peptone, or amino acids, recommended for the preparation of broth. While investigating blood, it was found that the serum proved a poor nutrient as compared with the cellular elements of blood, paralleling the findings with egg, in that the yolk was an excellent nutrient while the white proved to be a poor nutrient.[14] Likewise we found the addition of an inert diluent with practically no nutrient properties, such as agar added to an excellent nutrient, such as egg yolk, was only detrimental to the growth of the bacilli when planted in small numbers. Small amounts of agar might be added if desired, but the addition of nine parts to one part of a good nutrient proved ill advised. In a study of a nutrient made by adding fresh egg yolk to agar, it was found

that the inspissation of the yolk not only did not harm its nutrient qualities but enhanced it in contrast to the use of uninspissated yolk.[13] The non-sterile uninspissated yolk also had the disadvantage of possibly introducing into the findings extraneous bacilli such as among others the avian bacillus, which is known to be present at times in fresh eggs. From this and other studies, we concluded that the inspissation of egg mediums is not only beneficial but excludes the possibility of error in the findings of saprophytic acid-fast bacilli, which may grow like tubercle bacilli and confuse the results. The question is frequently raised as to whether mixing two good nutrient mediums such as egg yolk and potato possesses advantages.[13] In our experience we do not believe that there are any nutrient advantages to such mixing, although there appears to be no objection to the use of such mixture if it favors the individual's fancy. It must be recognized that the type of growth on potato, elevated and easily discerned, is different from that on egg, in which case the growth is of low wrinkled variety. Adding potato to egg favors the development of the elevated growth. What factor determines the character of growth we have not thus far disclosed, although it probably is dependent on the individual physico-chemical character of these complex nutrients.

Coincident with our studies on the nutrient requirements of tubercle bacilli, we became interested in a problem of like importance, that of finding a suitable reagent for destroying contaminations in pathological specimens without injuring small numbers of tubercle bacilli. Suffice here to say, however, that as a result of the study of a great many substances,[15] we concluded that an equal volume of 6% sulphuric acid or 5% oxalic acid at 37° C.,[16] intimately mixed with a suspected tuberculous specimen, for from one-half to one hour would destroy the contaminants without injuring small numbers of human or bovine tubercle bacilli present, and accordingly these reagents were recommended for this purpose. These reagents also were capable in control tests of destroying all varieties of acid-fast saprophytes in pure suspension which were available to us for testing.[17] It is interesting in this regard that acetic acid, which was also tried, would destroy the human and bovine tubercle bacilli without destroying acid-fast saprophytes or contaminants.

The foregoing will give you an idea of how a suitable method of culture was developed for growing small numbers of tubercle bacilli. Before closing, there is one feature of technical detail that may seem perfectly evident and yet is important to the success in growing these bacilli and might be said to have been developed over

the decades as a result of laborious trials and error. It seems almost unnecessary to touch upon this subject, but since the success in culturing depends greatly upon the elements of maintaining air and water balance in the tubes, it may be well to outline the means we pursue in obtaining this.[18] Almost all investigators in the past have used individual devices of their own. It is appropriate, however, to point out again that evaporation of the moisture from the medium will prove disastrous to the final success of growing tubercle bacilli. There is no means by which the moisture can be returned to the medium after it has been lost by evaporation. It is for this reason that every precaution should be taken to prevent such evaporation and especially so since the medium must be maintained in good condition for, at times at least, as long as three months or more. Therefore, all materials used to cover the tube must be moisture impervious and yet air pervious. In the early days, it was customary in various laboratories to cap the tube with tin foil, which proved an expensive method since the foil could be used only once. One would see also, in certain laboratories, a highly absorbent plug merely capped by a layer of paraffin through which a few pin holes had been made to allow air access. The cotton plug is a good bacterial filter and if it is impregnated by dipping into hot molten paraffin, maintains its properties as a bacterial filter and at the same time becomes impervious to moisture. Without an additional cover, water evaporates quite rapidly out of the tubes with only a paraffined cotton plug. To obviate this, tin foil or a paraffined cloth have been used by us in the past, and it appeared at the time fairly satisfactory. The paraffin cloth proved slightly better than the tin foil, both of which were held in place by a rubber band. The application of these devices proved time-consuming, and in testing out various methods, we found that the simplest and most time saving of all was to use an ordinary clean dry cork to stopper the tube after inserting the paraffined cotton plug. The cork must not be paraffined, because if this is done it becomes a hermetic seal and impervious to air. This provides a rapid easily applied means of preventing evaporation from the tubes, the combination of a well paraffined cotton plug with a clean sterile cork (not paraffined).

If we now examine 100 microscopic negative sputums, using the oxalic acid treatment and potato or egg glycerol medium as test culture mediums, and select these cases in a sanatorium, we find that from 54 to 57 of these yield positive cultures for tubercle bacilli.[19] This in itself speaks for the value and efficiency of the culture. In addition, we might add that when we compare the culture with guinea pig inoculation, the final results in positives are about the same, but the guinea pig with all its hazards is only a

presumptive test unless we check the lesions by finding acid-fast bacilli; while in the case of the culture, we possess colonies of tubercle bacilli ready and available for further tests if desired. It is needless to say that many more cultures can be housed proportionately for the same monetary outlay than guinea pigs. In concluding, I should like to summarize again the essential factors for obtaining growth from small numbers of tubercle bacilli based upon investigations pursued during the past several decades:

1. Growth Temperature—about 37.5° C. constantly maintained.
2. Absence of Light.
3. *Nutrient Medium*
   a. proper prepn. and sterility (30 minutes at 15 lbs. or 3 daily inspissations at 85° to 90° C. for 1 hour each).
   b. adequate nutrient qualities
      1. potato (neutralized); 2. egg yolk; 3. blood; 4. tissue.
4. Hydrogen Ion Concentration—about pH 7.0.
5. Isotonicity.
6. Maintaining Water and Air Balance.
   a. enough water in medium.
   b. paraffin impregnated cotton plug to prevent absorption of water from medium.
   c. cork stopper (tin foil or paraffin cloth may be used).
7. Elimination of Secondary Organisms from Contaminated Material by equal volume of
   a. 6% H_2SO_4 b. 5% oxalic acid for 30 minutes to 1 hour at 37° C., intimately mixed.
8. Absence of Inhibitory Dyes or Chemicals in the Medium (non-toxic or non-static dyes may be chosen to please the fancy and help in more readily discerning early colonies).

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LIMITATIONS IN THE RAPID TYPING OF PNEUMOCOCCUS BY THE NEUFELD REACTION

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Physicians are often asking the clinical laboratory for quick typing of sputum from cases of pneumonia. The idea is generally prevalent that this can be accomplished by the Neufeld micro "quellung" reaction, as suggested by Sabin, within a half hour after the specimen is received.

If the patient shows clinical symptoms of pneumonia and a report of the type is not given within a very short time, the laboratory may be exposed to criticism for delay.

It is true that in many cases the type can be determined by the micro method provided the following conditions prevail:

1. The sputum must be from a patient having pneumonia due to pneumococcus.

2. A sufficient number of pneumococci must be present in the sputum in order to give a positive reaction.

At the present time typing sera for types I, II, III and VII, and others, out of 32, are universally available. Serum for other types is under experimental preparation in some of the biological laboratories.

To illustrate what may be encountered in the typing of pneumococci, the following two cases will serve as examples:

CASE 1. A specimen of sputum of a man who had been ill for two days was obtained at the patient's home, with the request to have it typed immediately. The specimen was brought to the laboratory at 6 P. M. and consisted of a yellowish, somewhat tenacious sputum with faint traces of blood. A blood agar plate was streaked and a mouse inoculated with the sputum immediately. Then the Neufeld technic was carried out for types I, II and III sera with negative results. Very few bacteria of any kind were seen in the sputum when examined.

The physician was notified that type could not be determined by the micro method for lack of a sufficient number of organisms.

The following morning, fifteen hours after injection, the mouse appeared sick, it was killed and the peritoneal cavity washed out with sterile saline solution. Smear made from the mouse washings and stained for capsules showed many lancet-shaped encapsulated diplococci. The Neufeld Reaction gave positive results with type II serum. This was confirmed later in the day by test tube agglutination. The blood agar plate showed numerous pneumococcus colonies.

The sputum of this patient did not contain a sufficient number of pneumococci to enable typing by the rapid micro method; however, a diagnosis of type II pneumococcus was accomplished by the mouse and Neufeld methods in 15 hours from the time the specimen was received.

CASE 2. A man had been ill for five days when he developed bronchial symptoms. The physician requested a blood count, blood culture and sputum typed for pneumococcus. The sputum was very tenacious and of a deep dark red color due to large amount of blood present.

The specimen of this case was put through the same procedure as in the first case. A blood agar plate was streaked, and a mouse inoculated. The Neufeld Technic was carried out with negative results. The following morning the mouse was found dead, the peritoneum was washed out with saline solution. The material collected did not have the typical consistency of washings obtained from mice injected with sputum from pneumococcus pneumonia cases. There were flakes of mucus which did not break up completely upon shaking. Smear made of these washings stained for capsules showed large encapsulated bacilli; Grams stain revealed heavy, negatively stained bacilli surrounded by a distinct capsule.

The blood agar plate showed large convex, gray, moist mucoid colonies. The organism from these colonies was a Gram-negative bacillus and had the same morphological and staining characteristics as the bacillus isolated from the mouse.

The blood culture was positive and showed the same organism that was found in the mouse peritoneum and on the blood agar plate.

A diagnosis of Klebsiella pneumoniae (Friedlander's bacillus) was made.

From the experience of the two cases reported here it may be well to emphasize that a negative Neufeld Reaction does not exclude pneumococcus infection, nor pneumonia from other causes. However, quick typing results by the Neufeld Reaction with sputum from pneumococcus pneumonia cases can be obtained, provided a sufficient number of pneumococci are present of the type for which there is an anti-pneumococcic typing serum available.

A mouse inoculation and a blood agar plate culture should always be made for confirmation of the rapid typing method and for further studies if necessary.

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BLOOD GROUPS AND TRANSFUSIONS

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The existence of individual differences in human blood was first recognized by Landsteiner (1). He noticed that the serum of one human being could agglutinate the red blood cells of another normal individual. In 1900, he demonstrated the phenomenon of iso-hem-agglutination showing the presence of the iso-agglutinogens A and B in the human red blood cells and the presence of the corresponding iso-agglutinins a and b in serum. During the following year (2) he reduced this theory to a definite law and demonstrated the existence of three distinct human blood groups. Von Decastello and Sturli found the fourth and rarest type (3) Although Landsteiner was the pioneer in this field, we owe much to the men who furthered the work. Moss, Jansky, Guthrie and his co-workers, Coca, Klein, Levine and a host of others (4), (5), (6), (7), (8), have given us much useful data on the subject of blood groupings. Landsteiner and Levine have given us the properties M and N which are demonstrable by the use of immune agglutinins. These finer classifications are being pressed into service in the interest of medico-legal cases. As we are mainly interested in blood groupings in relationship to transfusions, we will not consider the sub-groups at this time.

Prior to the agitation for the newer classification known as the Landsteiner, which gives the groupings by letter, either the Moss or Jansky classifications have been used. This nomenclature by letter has been considered to have a very decided advantage since the group is identified by the agglutinable properties present in the red blood cells. This classification has also been known as the International classification of the special Health Committee of the League of Nations. The American Association of Immunologists also recognized it. In order not to be confused, until a uniform system of identification of blood groups is universally accepted, we have to consider the three best known groups and recognize each one. While the Jansky system has priority, the Moss classification has had more general use. These two differ only in placing the most common group. Where Moss uses IV to designate the most common class, Jansky numbers it I. The smaller group of Jansky is IV or the I of Moss. We must remember that there is no difference in the II and III groups of either Moss or Jansky. The Landsteiner method uses

the letters A and B to designate the agglutinins present in blood serum and the Greek letters alpha and beta for the agglutinogens of the red cells. Thus we have the groups A and B corresponding to II and III of both Moss and Jansky, AB with Jansky IV and Moss I, and O with Jansky I and Moss IV.

J. A. Kennedy (9) in a preliminary report in 1928 gives the results of a questionnaire sent to the leading hospitals of the United States and Canada to ascertain the classifications in general use and the opinions on changing to the Landsteiner Classification. From the replies it was evident that the Moss classification was the most popular system in use and that a change to the Landsteiner classification would probably lead to complications too great to warrant a change at that time. The final report in 1929 gave practically the same replies. About 70 per cent of the hospitals were not in favor of using the Landsteiner classification unless it became universal. However, due to the agitation, there probably are quite a few hospitals and physicians now who are using the Landsteiner and one or the other of the two favored classifications in making their reports of the blood groups of their patients and donors. The systems employed vary largely according to the locality, medical schools with which the physicians have been affiliated and, in some instances, with the technician employed.

Blood for grouping is collected in a 1 per cent or 2 per cent solution of sodium citrate in normal saline. The Moss test consists of mixing one drop of known type II serum with a drop of patient's red cell suspension on a cover glass and inverting over a hollow glass slide. Similarly a drop of known type III serum is mixed with a drop of patient's cell suspension and mounted. After 15 minutes any agglutination present is noted with the aid of the microscope. The same test can be performed using an open glass slide preparation and reading the results macroscopically. Sanford has used a method with dried type sera but this method has not been generally used.

Cross matchings can be made by a macroscopic test tube method described by Webster in his "Diagnostic Methods." This method, although very satisfactory because both agglutination and hemolysis can be noted, is apt to be too slow for some purposes. Either hanging drop or open slides can be prepared using donor's cells against recipient's serum and recipient's cells against donor's serum. Slides for these tests are prepared similarly to those for blood groupings. Readings can be made at the end of 15 and 30 minute periods.

False agglutination and Rouleaux formation must be carefully watched to avoid incorrect typings. The exact cause of rouleaux

formation is not definitely known. "This hyperviscosity of the cells," according to Webster (10), "has not at the present much clinical significance, but may be shown to be of importance when our knowledge concerning the normal viscosity of blood becomes more extensive." Levine (11) attributes this peculiar formation to a certain sticky substance in the serum of pregnant women or in patients suffering from certain infectious diseases as tuberculosis, pneumonia, and rheumatic fever. He claims this phenomenon to be characterized by rapid sedimentation of the red blood cells when observed in the test tube. Upon microscopic examination, this blood shows intense rouleaux formation which may closely simulate typical iso-agglutination. Diluting of the stock sera used in performing the test has been suggested to eliminate errors attributable to pseudo-agglutination. Slight rouleaux formation noticed on an open slide preparation can readily be broken up by rotating the slide or mixing the contents of the slide with an applicator stick or tooth pick.

The stock type II, III and IV sera should be frequently checked with known red cell types to detect a loss of agglutinating titer. In some laboratories the patient's cells are always checked with type IV serum as an added precaution against mistakes in blood groupings.

The existence of more than two iso-agglutinins, making more than four groups possible has been satisfactorily proven (14). This is a very interesting fact, especially when seen in the medico-legal aspect, yet its practical application is not far reaching. There is ample and valuable clinical proof that only four groups need be considered for routine purposes.

The theory of blood transfusions has been known since ancient times. Some students of the classics interpret certain obscure allusions of the Roman poets to illustrate that the knowledge of transfusions was practiced in the time of Augustus. They cite a quotation from Ovid to prove this assertion. It has been stated that the earliest application of the procedure was in the case of one of the later 15th century Popes. A Jewish physician claimed he was able to compound a life-saving medicine from the blood of youths. The three boys on whom he performed phlebotomy died. The arrest and subsequent escape of the practitioner caused quite a scandal.

It almost seems absurd to believe transfusion was practiced before Harvey's discovery of the circulation of blood in 1628. Sir Christopher Wren and Lower, of Oxford, experimented on transfusion from animal to animal in the 1660's. "Pepys' Diary" tells of Dr. Croone's experiment along this line.

In 1667 Denys of Montpelier applied the treatment to a young man who was weakened by repeated hemorrhages. The blood of a calf was used and the man recovered. About the same time, Pepys says a young man was hired by the Royal Society of England for 20 shillings. Birch's "History" states he was the first man in England to be transfused.

Miracles were expected from the use of transfusions. It was claimed to be a regular "Fountain of Youth." Many heated debates arose as to its merits and safety. Proof was furnished that it was extremely dangerous. Parliament of Paris issued an edict that consent of the Faculty of Medicine of Paris was necessary before a transfusion could be made. The practice of transfusion was condemned in 1739 by Heister in his "System of Surgery."

Now and then efforts were made to revive transfusion. It was not recognized as a legitimate procedure until after Blundell's Studies in 1825. In 1835, Bischoff was successful in completing a human to human transfusion, using defibrinated blood.

For years, some of the dangers of transfusion were known and for that reason it was only used in cases of severe hemorrhages which gravely threatened life. Both direct and indirect methods were used. Panum advanced the idea that blood should be defibrinated before injection.

The great perils were thought to be clotting and entrance of air into the veins. Heuter advocated injection of blood into an artery to lessen chances for air embolism. Infection was a grave danger before the days of Lister. In 1875 Landois discovered that animal serum was hemolytic to human blood. Because of Maragliand's observations in 1893 it was evident that human serum alien to another human serum was dangerous to use and might cause death.

It was not until about 1915 that transfusions were adopted by the medical profession as a satisfactory procedure. About that time Lewisohn suggested the use of citrate solution as an anticoagulant as it simplified administration. The World War did much to advance the use of transfusions.

A. M. Tiber (12) reviewed the statistics compiled on transfusions using a series of transfusion records covering a period from 1917 to 1929. In the cases reported by seven different authors, there were only twenty-two deaths resulting from a total of 10,242 transfusions. In a series of 4000 transfusions O. A. Brines (13) reported only two deaths directly attributable to transfusions.

In transfusions, as far as compatibility is concerned, the sole interest is that the plasma of the recipient does not agglutinate the cells of the donor. Group IV is theoretically the safest and most ideal because Group IV cells are not agglutinated by any agglutinins. A few weeks after birth, the groups become fixed and do not change. Because Group IV is usually secured more promptly, so-called universal donors are often used. There are many advocates for the use of these donors routinely. On the other hand, equally as many recommend their use with the reservation that universal donors be used only where cross matchings have been done and have proven satisfactory. There are others who will only use donors of the same type as the patient with the additional safeguard of perfect cross matchings. I have not been able to find a comparatively recent survey giving exact figures on the use of and preference for universal donors. At the present time it is a simple matter to secure competent donors because of the services of agencies, hospitals, etc., where persons having passed the required physical examination can be secured at short notice.

Transfusion reactions are most easily detected by an alert recipient and the transfusion can be quickly stopped before serious symptoms are produced. As little as 50 cc. of blood may give signs of causing cyanosis and lumbar pain. A reaction may be more severe with an unconscious or semi-comatose recipient before the operator realizes the situation.

There are various reactions and various causes for them. Some of these reactions are a simple rise in temperature; chills and chilly sensations; chills and rise in temperature; petechia; petechia with ecchymosis and hemoglobinuria; and allergic reactions.

Reactions are due mainly (1) to direct incompatibility caused by faulty blood grouping, (2) chemical reactions because of the anti-coagulant or unclean apparatus, and the (3) anaphylactic and allergic types of reactions. Some other causes for reaction are the too rapid injection of blood; the injection of too large an amount of blood; poor technic in performing the operation; and the presence of the early products of coagulation.

The first cause of reaction to be considered is that due to incompatibility after faulty blood grouping. Our so-called cold agglutination explains some of these reactions. These are not often encountered because cold agglutinins are rarely present under conditions at which ordinary typings are done—i.e., room temperature. Perry and Brunner (15) report a case of faulty blood grouping due to auto-agglutination. This reaction is defined by Boxwell and

Bigger (16) as "the clumping of erythrocytes into irregular masses visible to the naked eye, occurring in the presence of the individual's own serum, without bacterial action, at air temperature and reversible at body temperature." These auto-agglutinins can be freed from the cells by washing and suspending the patient's cells in warm normal saline solution in which solution they can be demonstrated. The case reported had the cells collected in the usual manner and typing made routinely. The patient was classified as a group I of Moss. However, when the patient's serum and cells were cross matched with donor's serum and cells of the same type, the patient's cells were agglutinated by the donor's serum at room temperature. The patient's red cells were then washed in normal saline and resuspended. The re-grouping then showed a new picture—the patient being reclassed as Moss IV. In this case a re-cross matching using a type IV donor showed no reaction. Because of the extreme anemia of the patient, the citrated cells contained an unusually large amount of serum. The agglutination might have been attributable to the auto-agglutinins that the patient's red cells had absorbed from the serum at lower than body temperature.

The second and most common cause of reactions is that due to the anti-coagulant used or to a chemical contaminator from the rubber tubing or other parts of the transfusion apparatus. Chills are the most common reaction due to this cause. Brines (17) is representative of many physicians who maintain that the use of injections of unmodified blood is rarely followed by chills. It is the contention of Meleny and his associates (18) and Lewisohn (19) that chills follow transfusions of non-citrated blood in the same proportion as they do following the use of citrated blood.

Lewisohn and Rosenthal (20) recommend that citrate and normal saline be made very carefully with triple distilled water. They further recommend that the appartus be taken care of in the following manner:

1. Apparatus should be taken apart after each transfusion and thoroughly washed in cold water.
2. Wash in a 1 per cent solution of cresol in green soap.
3. Follow with a thorough rinsing in tap water.
4. Boil parts for 5 minutes, needle for only 3 minutes, in a 0.1 per cent solution of sodium hydroxide.
5. Wash in distilled water to remove sodium hydroxide.
6. Rinse in triple-distilled water.

The apparatus is now ready to be sterilized. The glassware and rubber tubing are always boiled separately.

Karsner (21) explains the anaphylactic type of reaction as that in which there is a perfect blood typing and cross matching with no evidence of hemolysis in the urine. This type appears about one hour after transfusion and consists of high temperature, cyanosis, rapid respirations, mental confusion and convulsions. The character, severity and duration of the reaction, absence of incompatibility and of allergic skin manifestations usually serve to classify this type of reaction.

The knowledge of the mechanism of the anaphylactic reaction is rather vague. It has been suggested that agglutination and degeneration of platelets might be factors. Doan (22) believes many of these reactions are due to incompatibility of the white blood cells. If one believes this type of reaction is a true anaphylactic shock, the suggestion presents itself that a test for serum sensitization might be made before each transfusion. The rare occurrence of such phenomena would probably serve to discourage the conscientious application of such a test.

The role that food allergy might play in causing such reaction has led Brem, Zisler and Hammack (23) to experiment with fasting donors with favorable results. The recipient should be carefully questioned as to allergic effects and the donors for such patients be fasting. The reactions attributed to allergy are often mild and delayed and for this reason can not always be satisfactorily explained. Littlefield (24) reports an interesting case where the reaction was traced to the egg eaten by the donor just before the transfusion. The same author also reports the case of a patient who became sensitive to the blood of a donor who had been used several times in succession for the same patient. Cross matchings before each transfusion had been satisfactory. E. Traum (25), after a review of the literature on the subject, recommends different donors for repeated transfusions on the same patient.

Although the dangers of immediate shock and reactions must be given primary consideration, the more lasting result of directly transmitting diseases to the recipient must not be overlooked. There are cases on record where syphilis had been accidently acquired through transfusions where the blood of the donor had not been examined. Morgan (26), in a paper before the Association of American Physicians last month, analyzed the data and related the information thus obtained to our knowledge of the human and experimental disease. He cited 16 cases of "transfusion syphilis" that had been previously reported. We now use the Wassermann test—if time permits. For more rapid diagnosis, the Eagle flocculation test, the Kahn precipitation test and the Kline test for

syphilis and other similar tests are our greatest aids in preventing such calamities. In most instances, athough time is a factor in promptly performing transfusions, one or the other of these serodiagnostic tests for syphilis should be done.

Since the beginning of Landsteiner's work, a great host of scientists has worked with the theories of blood grouping and compatibility of donor with recipient. They have designed a less complicated apparatus and have perfected a simpler technic of administration. The fear of many complications has been removed entirely. The giving of blood from a healthy individual to a patient either as an immediate life-saving aid or as a hemopoietic stimulant has become an ordinary procedure to which we have become accustomed and which we now know is as safe as any of our more common minor surgical operations.

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THE PREPARATION OF TRANSUDATES FOR HISTOLOGICAL STUDY*

By PHYLLIS STANLEY, A.M., M.T.

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There are references in the literature describing the preparation of transudates for histological diagnosis, but they have not as yet found their way into text books of laboratory technic. For this reason, not many laboratories utilize these methods of diagnosis. Therefore, I think it is worth while to bring to your attention a simple method which we have developed in our laboratory.

In order to obtain the best results, it is necessary to have the co-operation of the doctor and nurses who are in charge of the thoracentesis or paracentesis. All of the specimen should be sent to the laboratory as quickly as possible after it is removed. Very often only a small amount of the transudate is sent or the fluid is kept for some time before being sent to the laboratory or more often the fluid is thrown away. As soon as the fluid is received, note is made of its general appearance, i.e., color, consistency and amount. Some of the fluid is poured into 250 cc. tubes and centrifuged at high speed for fifteen minutes. The supernatant fluid is poured off and more of the specimen is added and centrifuged. This process is repeated until all the sediment has been condensed into two or four of the tubes.

If the fluid arrives at a time when this step cannot be carried out, we add ten per cent formalin, equal volume and leave until ready to centrifuge. If there is not an opportunity to centrifuge all the specimen at once, or the quantity is too large to do all within two hours, then an equal amount of 10 per cent formalin is added to the portion which cannot be done immediately. If the formalin is added as soon as the specimen arrives in the laboratory, the cells are fixed and do not lose their characteristic appearance or become distorted. If a fluid is withdrawn at a time when there is no one in the laboratory to take care of it and no formalin is available to be added, the specimen can be put in the refrigerator for a few hours without autolysis.

After the sediment has been concentrated in the 250 cc. centrifuge tubes it is washed with ten per cent formalin into two fifty cc.

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round bottom tubes and then transferred into two small flat bottomed tubes. Ten cc. homeopathic vials are suitable. The sediment is centrifuged with the ten per cent formalin, and allowed to pack on the bottom. Some fluids form distinct layers at this time. The sediment will appear to have different types of cells, arranged in horizontal layers. The formalin is left on the sediment for one or two days until it is hardened. It is then centrifuged to make sure it is well packed and the formalin decanted. 70 per cent alcohol is added and left for about 24 hours. The two tubes are centrifuged and this step repeated with 95 per cent alcohol. Absolute alcohol is added and the tubes placed in the paraffin oven at 55° C. for about three hours. The alcohol is changed once or twice. Chloroform is then added and the sediment will rise to the top of the fluid. I usually stir around the edge of the tube with a teasing needle to loosen the sediment so that it will separate in one block and not break up. The tubes are left in the chloroform in the paraffin oven for about half an hour. The chloroform is drained off. If the sediment block has broken up, a small piece of gauze is tied over the top of the tube before it is inverted. Paraffin is then added, and changed every half hour for two and one-half hours. When the last change of paraffin has been added the tubes are left in the oven for five minutes before being cooled, so that if any small particles or cells may have separated in pouring off the paraffin they will settle to the bottom. To harden the paraffin block take the tube half full of paraffin and rotate slowly under cold running water, cooling all sides of the tube equally. As soon as a crust has formed over the top fill the tube with water and place in an ice water bath or in the freezing unit of the refrigerator.

To mount the block, heat the tube very gently over a small flame to loosen the paraffin slightly from the sides. Then wrap the tube in a piece of cloth and tap it with a hammer. Best results are obtained if the empty half of the tube is broken off first. The glass tends to shatter so that it can be picked off the paraffin mold without trouble. Now we have two small round blocks with the tissue embedded in them. One block is trimmed as it is, and mounted. The other block is cut in half vertically and mounted with the cut flat side down. When this one is cut, we will have a cross section of the layers (as previously mentioned).

A CONVENIENT MODIFICATION OF THE PIPET FOR DELIVERING KLINE ANTIGEN

By FRANCES REYNOLDS, B.A., M.T.

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Most workers are familiar with the microscopic slide precipitation tests for the diagnosis and exclusion of syphilis, as introduced by Dr. B. S. Kline of Mount Sinai Hospital at Cleveland. For this reason, the author will not attempt to go into the method as a whole, as the point of this article is one of technical detail only.

In his method of procedure (Journal of Laboratory and Clinical Medicine, October, 1930), Dr. Kline describes a pipet for transferring the antigen to the slide. He suggests the use of a Wright pipet made from glass tubing 6 to 10 mm. in diameter with the dropping end of the tube about 0.5 mm. in outside diameter, delivering a drop equal to about .008 cc. (62 drops per 0.5 cc.).

When we began doing the Kline Exclusion test in our laboratory, we made and used such a pipet. However, we found it had several disadvantages, namely, the finished product was of necessity so long and fine that it was too fragile to last very long; and each new pipet had to be adjusted carefully to make it deliver 62 drops per 0.5 cc. For these reasons, the author devised a modification which is in the form of a slender, glass syringe, with rubber bulb, and fine hypodermic needle. The beveled end of a No. 24 needle was cut off and the needle fitted to a ground glass tipped glass tube. Filling and delivering is controlled by using an ordinary medicine dropper bulb. The length of the whole syringe is about 5 inches.

EDITORIALS

THE IDEAL TECHNICIAN

Once upon a time medicine was practiced only by the doctors. Then specialization began to develop in medicine with the coming of new instruments and new devices and also with advancement of medical knowledge. Nurses became an adjunct to the medical profession after Florence Nightingale established nursing as a distinct profession. Soon physicians found that special training and special technic required for many of the practices in medicine might well be in the hands of persons especially trained for limited purposes. As a result technicians began to accumulate and today medicine is practiced not only by 150,000 doctors but by a vast number of technicians, including radiologic technicians, clinical technicians, laboratory technicians, physical technicians, physcal therapy technicians, anesthetists, dietitians, allergic technicians, and a host of others.

Obviously, in a profession that has grown as rapidly as has that of technician in the field of medicine there is a wide range of variability in training and accomplishment. There have been laboratory technicians, trained simply under the guidance of some physician in his own office. There are some who are the products of four weeks', eight weeks', or thirteen weeks' schools; others with one year of training in a hospital; some with two years of training in special technological schools and some with four years of training with a bachelor's degree. Some of the courses take the technicians simply through blood counts, urinalysis and basal metabolism determinations; others have departed into the realms of blood chemistry, roentgenology and physical chemistry.

Out of this evolution come some obvious facts. The rapidity of the development of medical science and the increasing importance of the adjuncts in the ways of machinery and chemistry have made the technicians a necessity.

There must be some type of standardization of education of technicians so that they may be suitably impressed with the ideals of scientific medicine, the ethics of the profession and the impor-

tance of attempting to achieve perfection in their work. A realization by technicians of the professional character of medicine will keep them away from the dangers of unionization and impress them with a realization of the position which they must occupy in the medical field. Particularly important is the establishment of some type of entrance qualification for the field which will be enforced by the hospitals and the medical profession. Suitable attention to these matters during the period of development of medical technology as a profession will avoid a great deal of grief and disappointment for the future.

MORRIS FISHBEIN, M.D., *Ed. J. A. M. A.*

TUBERCULOSIS

One of the greatest plagues to ever settle upon mankind still roams the world, reaping its harvest of lives from all ages, all classes, all creeds, and all races.

The ancient tombs and pyramids yield bodies preserved adequately enough that the modern X-ray equipment can reveal in some of them positive evidence of tuberculosis. By this means alone, disregarding other historical data, we may say that tuberculosis has infected man for approximately three or four thousand years. When one considers this period of time and then the comparatively short time since tuberculosis was recognized as a disease having a definite causative factor, the progress attained in treatment is indeed remarkable.

The Medical Technologist today has a greater opportunity to aid in the warfare against tuberculosis than ever before. So great is the demand for the various tests, conducted in modern sanatoria, that few procedures are exempt. Aside from the tests directly pertaining to tuberculosis, many tests arise from secondary conditions which follow in its wake.

Scientists in every nation are striving continually to solve this problem that has perplexed the greatest minds the world has known. The constant vigilance of health departments and physicians, with modern diagnostic agents, have caused treatment to be given many incipient cases in addition to many that are classed as open.

Rest and surgery, with proper diet and environment, are accomplishing wonders in restoring many patients, in a sound state of health, to society. With full recognition of the advances attained thus far, we must realize that the battle has not been won. We hope that the Medical Technologist of today utilizing the priceless volumes of scientific data, while co-operating with those in charge, will avail himself of that which opportunity offers.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS
OF THE
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

During the month of August, 6500 hospitals throughout the United States and Canada were circularized by the Registry. Attention was called to the rules and regulations of the Board of Registry and the co-operation of hospital administrations was requested in carrying out the program to elevate the scientific status of Medical Technologists, by encouraging their eligible laboratory personnel to apply for a certificate of competence. The hospitals were also asked to discourage training of students by technicians except under direct supervision of a clinical pathologist under the minimum requirements laid down by the Board of Registry.

Clinical Pathologists were also addressed and a copy of the new (sixth) edition of the Registry booklet was sent to them for their own information.

Each of our certificate holders received a copy of the latest edition of the booklet so that they may be familiar with the present requirements, and regulations governing the conduct of the Registry and impart this information to prospective applicants.

The Educational number of the Journal of the American Medical Association for August 29, 1936, carried an accredited list of training schools for Medical Technologists.

The Council on Medical Education and Hospitals of the A. M. A. is now jointly assuming the approval of teaching institutions in conjunction with the Board of Registry of the A. S. C. P. following the official acceptance of the standards set up by the Registry by the House of Delegates last May, in Kansas City.

The April examinations which were held throughout the United States and Canada brought 283 new certificate holders under the banner of the Registry. There were 37 unsuccessful candidates out of the 310 who appeared for the tests.

The following questions were used by the Examining Board in the written examination:

QUESTIONS FOR WRITTEN EXAMINATION, APRIL, 1936

General

1. (a) Name 6 important texts or manuals on clinical laboratory methods.
   (b) Name 10 important examples of clinical materials which are frequently submitted to the clinical laboratory for examination and tests. (Do not describe technique of any test.)

2. Describe the technique of a pregnancy test.

Clinical Bacteriology

3. (a) How many types of pneumococci (Diplococcus pneumoniae) are recognized at the present time?
   (b) Describe the technique of the Neufeld reaction used in typing pneumococci.
4. (a) Name 5 organisms which may be found in spinal fluid from cases of meningitis. What organisms is the cause of epidemic meningitis?
   (b) Describe technique employed in examination of spinal fluid from case of epidemic meningitis.

Hematology

5. Give the procedure for determining the following:
   (a) Color Index
   (b) Volume Percent
   (c) Icterus Index
   (d) Reticulocyte Count
   (e) Coagulation Time
6. (a) What is the color index if the Hb equals 20% and the red cell count is 800,000 cells per cu. mm.?
   (b) What is the normal ratio of white cells to red cells in human blood?

Blood Chemistry

7. State in what procedures the following reagents are used:
   (a) Nessler's solution
   (b) Alkaline copper tartrate solution
   (c) 10% sodium tungstate
   (d) Folin's phenol reagent
   (Do not describe technique or methods of preparation)

Urinalysis

8. Describe the qualitative and quantitative tests for albumin.

Tissue Technique

9. Name reagents employed in routine staining and mounting of tissue section, and state purpose involved in transfer of specimen through each procedure.

Serology

10. (a) List the material used in the Wassermann test. Upon what principle is this test based?
(Do not describe amounts used or technique but list the materials and state briefly the principle involved.)
(b) Name 3 other laboratory procedures used in the diagnosis of syphilis.
(Do not describe technique.)

NATIONAL

Roy R. Kracke, M.D. of Emory University, Georgia, and a member of the Board of Registry, is now the President of the American Society of Clinical Pathologists.

Prof. John Scott Haldane, F.R.S., physiologist and philosopher, has died at his home in Oxford at the age of 75. It was between 1892 and 1900 that he introduced methods for determining the amount of hemoglobin in the blood, the volume of the blood, and other important findings.

CANCER: In the Connecticut State Department of Health a physician has been appointed in a newly created position, to specialize in cancer work. A state survey with statistical studies of such cases is being made: about sixteen clinics are already operating at general hospitals.

ROCKY MOUNTAIN SPOTTED FEVER: The Illinois State Health Department has reported two cases, and in the District of Columbia two children have been treated for this disease recently.

RABID DOGS: In a certain district in Detroit two rabid dogs were reported during April, six during May, and three to June thirteenth. The Health Department urged vaccination of all dogs within this area. Up to June thirtieth of this year in Illinois, 4,374 cases of dog bites had been reported and large quantities of vaccine distributed.

PLAGUE: The U. S. Public Health Service of Nevada has recently reported the identification of plague infection in ground squirrels shot in Alko County.

A case of visceral Leishmaniasis has recently been found in a boy in Rio de Janeiro. The parasites were found on slides prepared from the patient's blood removed by puncture from the spleen.

Awards

Dr. Bernard L. Wyatt, former director of the Desert Sanatorium and Institute of Research, Tucson, Arizona, the James E. Stacey Award consisting of a medal and an honorarium of $100, from the University of Cincinnati College of Medicine, for significant contribution in the field of focal infection.

Dr. Eduard Uhlenhuth, Ph.D., of the University of Maryland School of Medicine, Baltimore, Md., the Van Meter Prize of the American Association, for the Study of Goiter.

Dr. Karl Fischel, Saranac Lake, N. Y., the Merrit H. Cash Prize for his essay on "The Problem of Tuberculosis," from the Medical Society of the State of New York.

Dr. Arthur J. Bedell, Albany, N. Y., the Lucian Howe Prize for his essay on "Choroideremia."

Dr. James Ewing of Cornell University, N. Y., the John Scott Award consisting of a bronze medal and $1,000, for research in classifying tumors, from the Philadelphia Board of City Trusts.

Gifts

Sir Herbert Austin, automobile manufacturer, has made a gift of $1,250,000 to the famous Cavendish Laboratory of Cambridge University. This laboratory perpetuates the name of Henry Cavendish who discovered the composition of water. Other advances made in this laboratory were the discovery of the election, 1897, and the neutron, 1932. Of remarkable apparatus in use there is Prof. C. T. R. Wilson's cloud chamber in which the tracks of atomic particles have been photographed, and D. F. W. Aston's mass spectrograph by which individual atoms may be weighed.

BOOK REVIEWS

PARENTERAL THERAPY. A Ready Reference Manual of Extra-Oral Medication. By Walton Forest Dutton, M.D., Formerly Medical Director, Polyclinic and Medico-Chirurgical Hospitals Graduate School of Medicine, University of Pennsylvania; Visiting Physician to the Northwest Texas Hospital; Visiting Physician to St. Anthony's Sanitarium; Director, Medical Research Laboratories, Amarillo, Texas; Colonel, Medical Officers' Reserve Corps, U. S. A., and George Burt Lake, M.D., Formerly Special Lecturer in Hygiene, Purdue University; Editor, Clinical Medicine and Surgery; Associate, American College of Physicians; Educational Lecturer, Illinois State Medical Association; Colonel, Medical Officers' Reserve Corps, U. S. A. Charles C. Thomas, Publishers, Springfield, Ill., 1936. Pp. 376, Price $7.50 postpaid.

This unique publication is divided into three parts. Part I deals with the technique of parenteral therapy and diagnostic needle punctures. In the introduction various factors influencing medication are discussed such as quality and quantity of drug administered, solubility, individual variation and age of patients, viscosity, blood chemistry, etc., etc. The next chapter is devoted to materials and equipment and their care. The technique in detail is described in the following chapters for intradermal, hypodermic, intramuscular, intravenous and intraperitoneal injections; blood transfusion, methods for collection of blood and serum, intracardiac and intrapericardial injections; pneumothorax, intraspinal serum injections, cisternal and intraventricular punctures. Various forms of anaesthesia are aptly described—intravenous, infiltration, caudal epidural and spinal. Inhalation and absorption anaesthetics and their technique of administration are given in detail. The chapters on alcohol injection for local nerve block and iodized oil injections for certain painful conditions of the locomotor and nervous systems contain a wealth of valuable information. The injection treatment of varicose veins, hemorrhoids, herniae, hydroceles and bursae are adequately described.

The authors' cautions regarding dangers and pitfalls of various injections, and safe limits of dosage are particularly to be commended as novices should not attempt some of the procedures such as nerve and ganglia injections with alcohol, spinal anaesthesia and

the like. Individual exceptions may be taken to some of the details of technique but this may be a matter of personal difference. In general the technical procedures are given very well. On page 115 the statement that "the dose of anaesthetic should be regulated, according to the drug, for each 15 pounds of the body weight of the patient" should be amplified and dosages given and also more space devoted to the relative advantages and disadvantages of the preparations used for spinal anaesthesia. The just criticism should be made that a certain make of syringe is frequently mentioned and no other. There are other syringes that are equally as good.

Parts II and III consist of a therapeutic index and pharmacologic notes. This feature alone is worth the price of the book. I have several drawers full of advertisements for numerous parenteral drugs from various manufacturers which I had intended to index in some manner. I think now I shall dispose of the whole lot of them into several waste paper baskets and use the book for a ready reference manual which has them indexed in alphabetical order and probably contains all that I have and many more.

Undoubtedly this book would be a profitable addition to the office library of any physician, whether general practitioner or specialist, and contains much valuable information for the laboratory technologists.

DISORDERS OF THE BLOOD. Diagnosis, Pathology, Treatment and Technique, by Lionel E. H. Whitby, C.V.O., M.C., M.A., M.D. (Cantab.), F.R.C.P. (Lond.) D. P. H. and C. J. C. Britton, M.D. (New Zealand). Published by P. Blakiston's Son & Co., Inc., Philadelphia. Water and vermin proof cloth binding, 543 pages. Price $7.00.

This is indeed a well written volume. The authors have reduced to reasonable compass a great portion of haematological literature of the past ten years, in some instances quoting phases that are somewhat controversial and treating them in an unbiased manner. A number of references are given with the conclusion of each chapter, some of which are key papers. Eight color plate illustrations are given.

The origin, development, functions and fate of the blood cells is discussed in Chapter I. Succeeding chapters progress in an orderly manner discussing haematological diagnosis in its many phases including the physical and chemical properties of blood cells and plasma. The causes of anaemia are then discussed, followed by a study of haemopoietic substances and their action.

The greater part of the book is given to the study of the many types of anaemia, giving in most cases, the aetiology, symptoms, diagnosis and methods of treatment. Haemagglutination, blood transfusions, blood grouping, allergy and pathological conditions of various origin are discussed at length. The final chapter is devoted to the mechanics of the technical phase of blood study in general. A subject and separate author's index complete the book.

Briefly, one can say this is a valuable book for the haematological student, for the technologist in the clinical laboratory, and for the physician. A veritable well of knowledge, fed by experience and reference.

ABSTRACTS

BLOOD CALCIUM DETERMINATION, USING STANDARD CALCIUM CHLORIDE SOLUTION: Israel Schwartz, Phar.D., F. A. I. C., Jour. Lab. and Clin. Med., Vol. 21:4, January, 1936.

The prepared standard calcium chloride solution used in this method keeps indefinitely, which is a decided advantage over the deterioration of the usual standard potassium permanganate solution. The potassium permanganate solution used in this procedure, need not be standardized, and the other volumetric solutions heretofore employed are dispensed with. The procedure is simple, and the results accurate and dependable.

A DIRECT MICROTITRATION METHOD FOR BLOOD SUGAR: Miller and Van Slyke, J. Biol. Chem. 114:583, July, 1936.

0.1 c.c. sample of blood is deproteinized with Cd $(OH)_2$, the excess of which is removed with $BaCO_3$. The filtrate is heated with excess ferricyanide and the ferrocyanide so produced is titrated with ceric sulfate, using setopaline C as indicator.

A MODIFIED STAIN FOR STIPPLE CELLS IN LEAD POISONING: Ruth A. McKinney, Ph.D., and Saul Rosenzweig, M.D., Detroit, Mich., J.A.M.A., May 9, 1936, Vol. 106, No. 19.

With this method it is possible not only to count the stipple cells but also to make the usual differential count of the white blood cells at a single examination. Also with this technic dried smears two months old may be stained as satisfactorily as fresh ones.

METHOD OF STAINING PROTOZOA IN BULK: Wm. S. Stone, , M.D., Washington, D. C., J. Lab. and Clin. Med., May, 1936, Vol. 21, No. 8.

This procedure differs from other standard hematoxylin staining methods only in that the organisms are not fixed and stained on slides or cover slips, but in bulk. The organisms are concentrated and then carried through the steps of fixation and staining in 50 cc. centrifuge tubes. Very few organisms are lost during fixation, staining, and mounting. The organisms are natural and lifelike and not distorted by the reagents or manipulations used.

A METHOD FOR MEASURING THE FRAGILITY OF ERYTHROCYTES IN SALT SOLUTION: Richard T. Beebe, M.D., and Edw. P. Hanley, A.B., Albany, N. Y., J. Lab. and Clin. Med., May, 1936, Vol. 21, No. 8.

This article gives technic for newer, and possibly more accurate method for measuring resistance of erythrocytes to salt solution. A stock solution of 0.5% salt solution is used for the preparation of the other percentages and the material is measured in a buret to assure accuracy. Additional equipment consists of 15 cc. calibrated centrifuge tubes and a dropping pipette. Oxalated venous blood is used, 1 cc. to each tube containing 5 cc. of saline in the varying strengths. After standing in ice-box for twenty-four hours, tubes are centrifuged and results read by the hemolysis produced, but a more accurate index is obtained by measuring the rate of decrease in volume of the column of packed erythrocytes.

DIAGNOSIS OF CARCINOMA OF STOMACH FROM A FRAGMENT OF TUMOR OBTAINED DURING GASTRIC ANALYSIS: Louis G. Jekel, M.D., St. Louis, Mo., J. Lab. and Clin. Med., May, 1936, Vol. 21, No. 8.

This article demonstrates forcibly the importance of searching the gastric contents for possible particles of cancer tissue. Early diagnosis of cancer may thus be made even when X-ray and other procedures show no pathological findings.

PRESERVATION OF ANTIGENIC SPECIFICITY OF SHEEP CORPUSCLES: Ashby, Winifred, Ph.D., J. Lab. & Clin. Med., Vol. 21, June, 1936, No. 9.

This article gives method for such a prolonged preservation of the antigenic specificity of sheep corpuscles that results by the Kolmer-Wassermann test were comparable to those with fresh cells by using sheep cells more than a year old. This preservation has been accomplished by diluting sheep blood, 1 to 10, with isotonic solutions of sucrose, dextrose, magnesium chloride, and sodium citrate, and maintaining the dilutions at 3 to 8° C.

SEDIMENTATION RATE AND RED CELL VOLUME: A Method for Determining in Infants and Children with the Use of Capillary Blood. Smith, Carl H., M.D., Amer. J. Med. Sc., Vol. 192, No. 1, July, 1936, No. 772.

The special tube which has been designed for this method represents a modification of the features of the Cutler method and also the Wintrobe tube used with small quantities of blood. The com-

plete apparatus may be obtained from E. Machlett & Son, 220 E. 23rd St., New York City.

PROTEUS X-19, PLEO-ANTIGENICITY OF: Welch, H., Mickle, F. L., and Borman, E. K., Am. J. Pub. Health, 24:1157, 1934.

On the basis of the findings enumerated in this paper, it is recommended that Proteux X strains used in the Weil-Felix reaction be checked at least once a month to insure working with a nonmotile culture and at least once each quarter to exclude spontaneously occurring variants.

WEIL'S DISEASE, Serological Diagnosis of: Brown, H. C., Brit. M. J., 1:411, 1935.

This article gives procedure for an adhesion test found as reliable as agglutination test for diagnosis of infection with L. ictohemorrhagicae. It is more rapid and easier to read. The reagents required: Patient's serum, young broth culture of L. icterohaemorrhagicae, saline suspension of culture of B. coli, fivefold dilution in saline of guinea pig's serum. Results read in thirty minutes.

POLYCHROME BLOOD STAIN, for General Purpose: Groat, Wm. A., J. Lab. and Clin. Med., Vol. 21, June, 1936, No. 9.

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TISSUE, Butyl Alcohol and Cytological Technic: Zirke, C., Science 80:481, 1934.

The following mixture is very useful for cleaning slides: Water, xylene, and N-butyl alcohol, each 1 part; ethyl alcohol, 2 parts. A few drops of N-butyl alcohol added to clouded xylene or alcohol in tissue staining jars will keep the solutions clear and useable. Proportions of the reagents are also given which will prevent precipitation and keep the solutions good for nearly 100 slides.

STAINING OF SPIROCHETES, SPIRILLA, SPERMATOZOA, AND RELATED ORGANISMS, A New, One-Minute Method for: Gelarie, Arnold J., M.D., J. Lab. and Clin. Med., Vol. 21, July, 1936, No. 10.

Six reasons are enumerated why Burri's India ink method is unsatisfactory, and Gelarie's technic is the outcome of an effort to overcome these obstacles. He states that numerous and exacting tests have been highly satisfactory. The organisms stain deep blue purple and are easily identified from the surrounding material.

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